

Synthesis of *o,p*-EDDHA and Its Detection as the Main Impurity in *o,o*-EDDHA Commercial Iron Chelates

MAR GÓMEZ-GALLEGO,[†] MIGUEL A. SIERRA,^{*,†} ROBERTO ALCÁZAR,[†]
 PEDRO RAMÍREZ,[†] CARMEN PIÑAR,[†] MARÍA JOSÉ MANCHEÑO,[†]
 SONIA GARCÍA-MARCO,[#] FELIPE YUNTA,[#] AND JUAN JOSÉ LUCENA[#]

Departamento de Química Orgánica, Facultad de Química, Universidad Complutense,
 28040 Madrid, Spain, and Departamento de Química Agrícola, Facultad de Ciencias,
 Universidad Autónoma de Madrid, 28049 Madrid, Spain

Ethylenediamine-*N,N*'bis(*o*-hydroxyphenyl)acetic acid (*o,o*-EDDHA) is one of the most efficient iron chelates employed to relieve iron chlorosis in plants. However, the presence of positional isomers of EDDHA in commercial iron chelates has been recently demonstrated, and among them, it has been claimed that ethylenediamine-*N*(*o*-hydroxyphenylacetic)-*N'*(*p*-hydroxyphenylacetic) acid (*o,p*-EDDHA) is the main impurity present in EDDHA fertilizers. Here we report the preparation of *o,p*-EDDHA, a compound whose synthesis had not been previously reported. The synthetic *o,p*-EDDHA is able to form ferric complexes, and it has been used as a standard in the analysis of the impurities of commercial iron fertilizers. The presence of *o,p*-EDDHA/Fe³⁺ in commercial samples has been unambiguously demonstrated by HPLC.

KEYWORDS: *o,o*-EDDHA; *o,p*-EDDHA; iron chelates; fertilizers

INTRODUCTION

Iron chlorosis is a nutritional disorder in plants that affects their development and decreases the yield of many crops. Chlorosis results in a decrease in the amount of chlorophyll and is manifested in a gradual disappearance of the green coloring of the plants (1, 2). The origins of this complex problem are diverse, ranging from nutritional disorders to infections caused by fungus, bacteria, insects, etc. Today, fertilization with synthetic iron chelates is the most common agricultural practice to relieve this problem, and ethylenediamine-*N,N*'bis(*o*-hydroxyphenyl)acetic acid (*o,o*-EDDHA) (1 in Figure 1) is among the most efficient iron chelating agents used (3). This compound has two phenol groups on a diaminocarboxylic acid backbone and is able to form ferric complexes (2a and 2b in Figure 1) of high stability in neutral and in alkaline solutions. The structural characterization of the Mg-(*rac*-Fe(III)-EDDHA)₂ salt shows that this class of chelate has an octahedral disposition, with the *o,o*-EDDHA ligand hexacoordinated to the Fe nucleus (4). The [6,5,6] arrangement of rings across the Fe-center having the phenolic groups in equatorial positions is considerably more favored than the alternative [5,5,5] arrangement. Nevertheless, it has been calculated that 0.5% of the [5,5,5] complex is present in the racemic mixture (5).

Most commercial iron fertilizers are based on ligands that have phenol groups on a polyamine–carboxylic acid backbone,

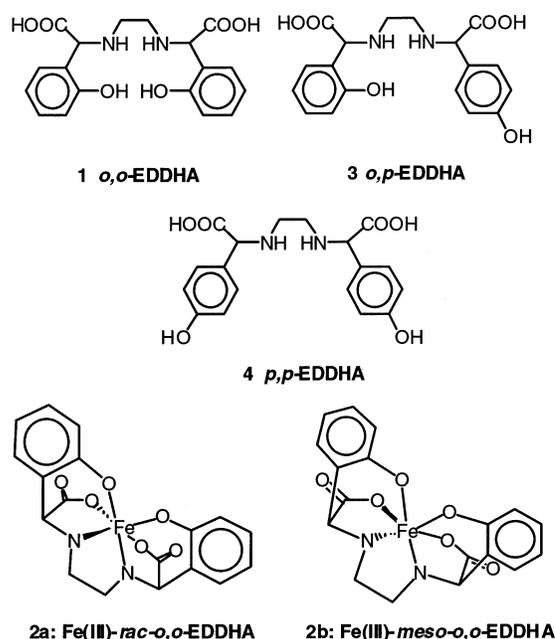


Figure 1. Chelating agents and chelates described in the text.

and several synthetic methods have been developed to obtain this type of compound. The synthesis of *o,o*-EDDHA (1 in Figure 1) originally reported by Kroll (6) in 1957, is a Strecker reaction on the imine derived from ethylenediamine and salicylaldehyde. Although the procedure can be employed to obtain other symmetrically substituted EDDHAs (7, 8), the

* Corresponding author. E-mail: sierraor@quim.ucm.es. Fax: 34-91-3944103.

[†] Universidad Complutense.

[#] Universidad Autónoma.

drawback is the need of liquid HCN during the industrial process. Other approaches to the synthesis of ethylenediamine-bis(*o*-hydroxyphenyl)acetic acids are based on a Mannich-like reaction between phenol (or substituted phenols), ethylenediamine, and glyoxylic acid (9, 10). This method is used for the preparation of all of the EDDHA currently in the market.

The use of commercial chelates has dramatically increased in the last years, not only in agricultural chemistry but also in other fields (2, 11, 12). Some analytical methods for the quality control of such products have been developed, but generally they have focused on determining the content of chelated metal in the product (13–15). However, other important aspects related to the purity of the ligands employed in the commercial formulations have been neglected. This is a very significant matter because, as we have commented before, *o,o*-EDDHA is prepared from ethylenediamine, sodium glyoxylate, and excess of phenol. This method produces mixtures of three regioisomeric products, namely, *o,o*-EDDHA, *o,p*-EDDHA, and *p,p*-EDDHA (1, 3, and 4, respectively, in Figure 1) in variable amounts. The lack of purity of commercial iron chelates of *o,o*-EDDHA (16) and the presence of positional isomers of the phenol group in commercial *o,o*-EDDHA/Fe³⁺ samples has been recently addressed by us (17).

It has been claimed that the main impurity present in *o,o*-EDDHA commercial iron chelates is the ethylenediamine-*N*(*o*-hydroxyphenylacetic)-*N'*(*p*-hydroxyphenylacetic acid (*o,p*-EDDHA) isomer (3 in Figure 1) (16, 17). However, as far as we are aware, the intentional synthesis and purification of *o,p*-EDDHA has not been previously reported, and hence, the presence of *o,p*-EDDHA as an impurity in commercial fertilizers has not been unambiguously confirmed to date. This fact excited our interest in the design of synthetic routes for the preparation of *o,p*-EDDHA and by extension to any other related unsymmetrically substituted chelating agents. This class of compounds cannot be obtained by any of the reported methods that are directed to the synthesis of symmetrical *o*-hydroxyarylacetic acid derivatives. In addition, the ability of *o,p*-EDDHA to form ferric complexes is unknown, and it would be interesting to determine their structure and properties, thereby establishing whether the iron chelates derived from 3 could be useful as fertilizers. This paper is a part of our ongoing research to establish the factors that affect the efficacy of pure and commercial iron chelates used with agricultural purposes. Here we report two complementary routes to prepare *o,p*-EDDHA 3. The procedures described below could be employed to synthesize other unsymmetrical ethylenediamine-bis(hydroxyphenyl)acetic acids, which opens the uses of chelates in agriculture and other fields to new possibilities. The obtained *o,p*-EDDHA will be used to confirm the presence *o,p*-EDDHA/Fe³⁺ complexes in commercial products.

MATERIALS AND METHODS

All the products obtained in the synthesis of *o,p*-EDDHA were characterized by spectroscopic techniques. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker 200-AC (200.13 MHz for ¹H and 50.03 for MHz¹³C) spectrometer. Chemical shifts are given in ppm relative to the corresponding deuterated solvent. IR spectra were taken on a Perkin-Elmer 781 spectrometer. Merck silicagel (230–400 mesh) was used as the stationary phase for purification of crude reaction mixtures by flash column chromatography. The synthesis of *o*- and *p*-methoxyphenylglycines was done following the procedure of Stein et al. (18). The corresponding methyl glycinates were obtained as hydrochlorides by the standard procedure, refluxing the amino acids with SOCl₂ in MeOH. Free methyl *o*- and *p*-methoxyphenylglycinates were obtained from their hydrochlorides by neutralization with saturated

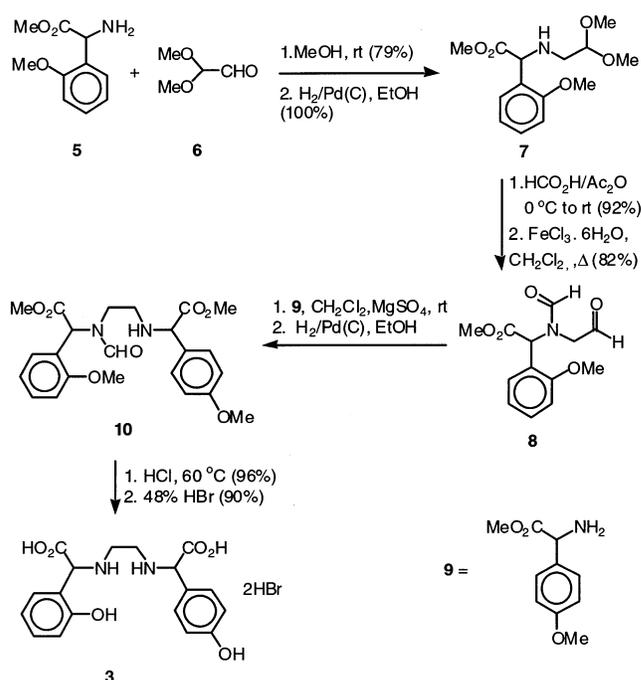


Figure 2. Synthetic route for *o,p*-EDDHA starting from arylglycinates and glyoxal.

NaHCO₃ solution followed by extraction with CH₂Cl₂ or generated in situ by reaction with Et₃N. Monoprotected ethylenediamine was prepared by a modification of the reported procedure (19). The full experimental data of all the compounds synthesized in this study are depicted in the Supporting Information.

The *o,p*-EDDHA product was evaluated for its ability to complex iron(III) and copper(II) by photometric titration and potentiometric titrations, respectively. The end points in both potentiometric and photometric titrations were calculated by the second derivate smoothed of the original data (20) and by Grant equation, respectively. The molar extinction coefficient for the iron chelate was also determined at 480 nm in order to compare it with that of the *o,o*-EDDHA.

For preparation of the *o,p*-EDDHA/Fe³⁺ complex, the chelating agent was dissolved in NaOH (1:3 molar ratio). Then, an amount of FeCl₃ that was calculated to be 5% in excess of the molar amount of ligand, was added. The pH was adjusted between 6 and 8 during the addition, and finally, the *o,p*-EDDHA/Fe³⁺ solution (pH 7) was left to stand overnight and then filtered through 0.45- μ m membranes. The solution of *o,p*-EDDHA/Fe³⁺ complex and a solution of a commercial Fe-chelate were compared using a Waters Symmetry C₁₈ 150 \times 3.9 mm column and an HPLC with a Waters 2690 Separation Module (Alliance). The absorption spectra (200–600 nm) were recorded with a Waters 996 photodiode array detector and Millennium 2010 chromatography data system. All commercially available compounds were used without further purification.

RESULTS AND DISCUSSION

We have designed two complementary routes to synthesize *o,p*-EDDHA. The first approach requires arylglycinates and glyoxal as reagents, whereas the second is based on the Strecker reaction starting from substituted benzaldehydes and ethylenediamine.

Prior to the synthesis, the feasibility of the different steps of the first approach was tested by preparing ethylenediamine-bis-phenylacetic acid starting from commercially available phenylglycine (Aldrich). The full experimental procedure is detailed as Supporting Information Material.

As the first approach to synthesize *o,p*-EDDHA, methyl *o*-methoxyphenylglycinate (5 in Figure 2) was employed as the starting material. Thus, equimolar amounts of glyoxal mono-

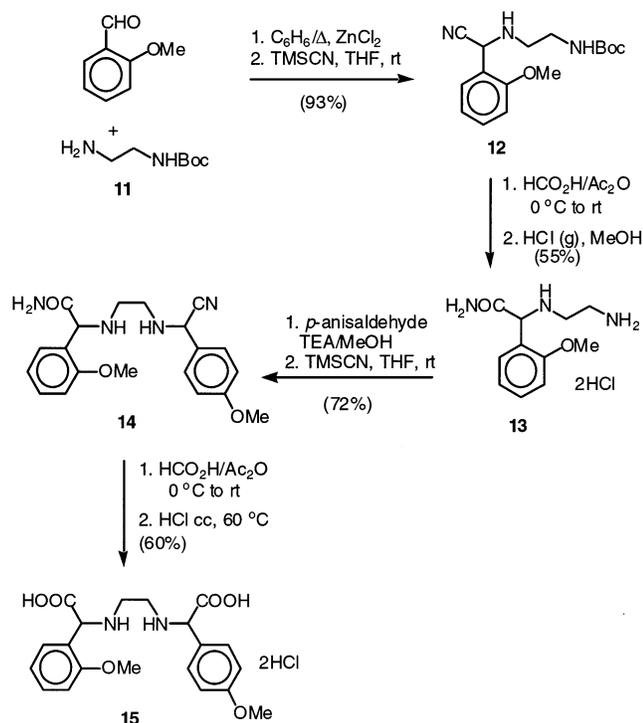


Figure 3. Synthetic route for *o,p*-EDDHA starting from substituted benzaldehydes and ethylenediamine.

acetal (**6** in **Figure 2**) (60 wt. % in H₂O) and glycinate (**5** in **Figure 2**) were reacted in dry MeOH, and the imine thus obtained (79%) was subsequently hydrogenated to form aminoacetal (**7** in **Figure 2**) in quantitative yield. Because of the known instability of α -aminoaldehydes (**21**), the formylation of the nitrogen atom (Ac₂O/HCOOH) (**22**, **23**) had to be effected prior to the deprotection of the acetal group in **7**. Treatment of the obtained formamide with FeCl₃·6H₂O, in boiling Cl₂CH₂ (**24**) yielded aldehyde (**8** in **Figure 2**) (82%) that was condensed with *p*-methoxyphenylglycinate (**9** in **Figure 2**) to give the expected imine in the next step. The imine was subsequently transformed into diester (**10** in **Figure 2**) by catalytic hydrogenation (65%, two steps). Finally, *o,p*-EDDHA was obtained from diester (**10** in **Figure 2**) by acid hydrolysis and further treatment with concentrated HBr. The best results were obtained by performing the sequential hydrolysis–deprotection of the methoxy group in this order. In fact, the removal of the methoxy groups in **10** with BBr₃ occurred, yet in very low yields (29%). Through this route, *o,p*-EDDHA was isolated as dihydrobromide and as a 1:1 mixture of diastereoisomers (**Figure 2**). The structure of the product is fully consistent with the ¹H and ¹³C NMR data.

The second approach started with the condensation between the mono BOC-derivative of ethylenediamine (**11** in **Figure 3**) and *o*-anisaldehyde. The resulting imine was reacted with trimethylsilyl cyanide (TMSCN) (**25**) in absence of catalyst, to form α -aminonitrile (**12** in **Figure 3**) in very high yield (93%). Formylation of the amino group in **12** (HCOOH/Ac₂O) followed by treatment with HCl (gas) in dry MeOH, resulted in the hydrolysis of the nitrile and the BOC groups, to give amide (**13** in **Figure 3**) (55%). This compound was next condensed with *p*-anisaldehyde to yield the expected imine (62%), which was transformed into nitrile (**14** in **Figure 3**) (72% yield) with TMSCN. Although Strecker adducts are generally stable under neutral conditions, they may undergo rapid decomposition under either acidic or basic conditions via a retro-Strecker reaction (**22**). To avoid this undesirable process, the hydrolysis of nitrile

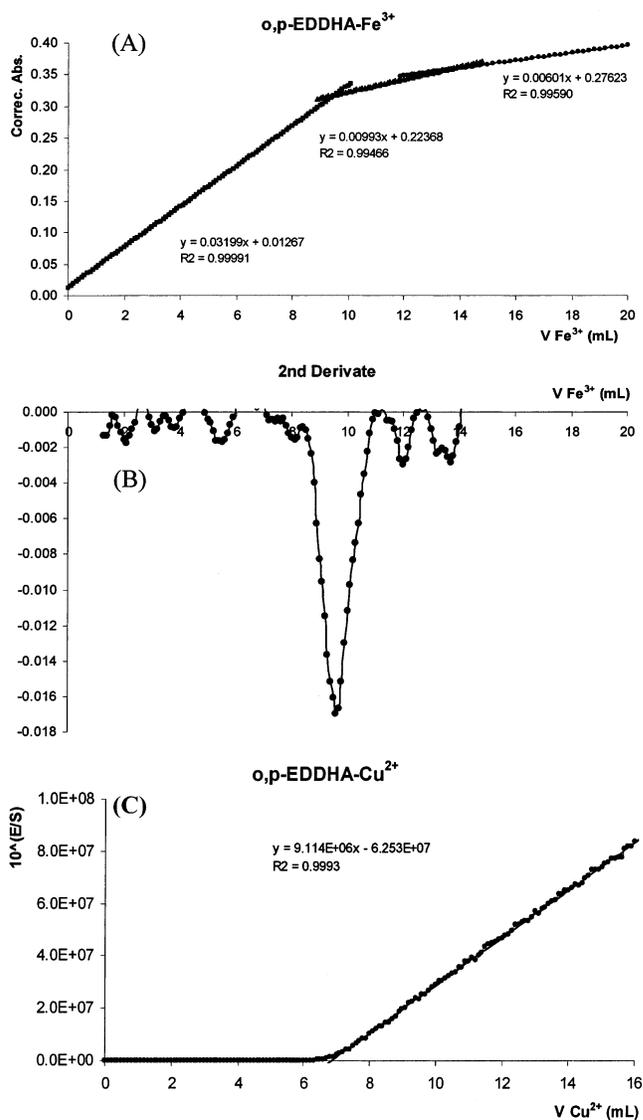


Figure 4. Photometric plot (A) where the end points have been calculated by the linear segments intersection calculated with the minimum of the second smoothed derivated (B). (C) Potentiometric titration with Cu(II) solution. Grant equation was used to calculated the end point.

14 was achieved by formylation of the amino groups and subsequent treatment with concentrated hydrochloric acid. In this way, *o,p*-methoxy-EDDHA (**15** in **Figure 3**) isolated as dihydrochloride, and as a 1:1 mixture of diastereoisomers was obtained in 60% yield. On the basis of ¹H and ¹³C NMR measurements, this compound was identical to that resulting from the hydrolysis of compound **10** (**Figure 2**) with concentrated HCl. Therefore, the two approaches to the synthesis of *o,p*-EDDHA **3** were successfully accomplished (**Table 1**).

The titrimetric purity of the synthetic *o,p*-EDDHA **3** obtained by potentiometric titration with Cu(II) solution (69.7%) is slightly higher than that obtained by photometric titration with Fe(III) solution (59.7%) (**Figure 4**). This difference could be due to the presence in the sample of a small amount of impurities having amino group, able to complex copper but not iron. Despite this fact, the photometric plot is very clean and clearly indicates that the product may be used as standard compound.

The molar extinction coefficient (ϵ) at 480 nm obtained for *o,p*-EDDHA/Fe³⁺ was 2130. This value is almost half of the ϵ observed for the *o,o*-EDDHA/Fe³⁺ complex at the same wavelength (4814) (**26**). At 480 nm, the light is being absorbed

Table 1. ^1H and ^{13}C Signal Assignments for Compound **3** in $\text{D}_2\text{O}/\text{Na}_2\text{CO}_3^a$

proton	δH	carbon	δC
CH (aromatics)	6.96–6.72 (4H) 6.54–6.34 (4H)	CH (aromatics) C ipso (aromatics)	131.2, 130.6, 129.9, 129.2, 118.3, 117.8 159.3, 127.0, 124.0
CHNH	4.17 (1H), 3.7 (1H) ^b	COOH CHNH	179.9, 177.7 66.9, 64.3
CH_2	2.56–2.39 (4H)	CH_2	45.4, 45.0

^a In units of ppm (^1H 200.13 MHz, ^{13}C 50.03 MHz). ^b Meso/racemic 1:1 ratio.

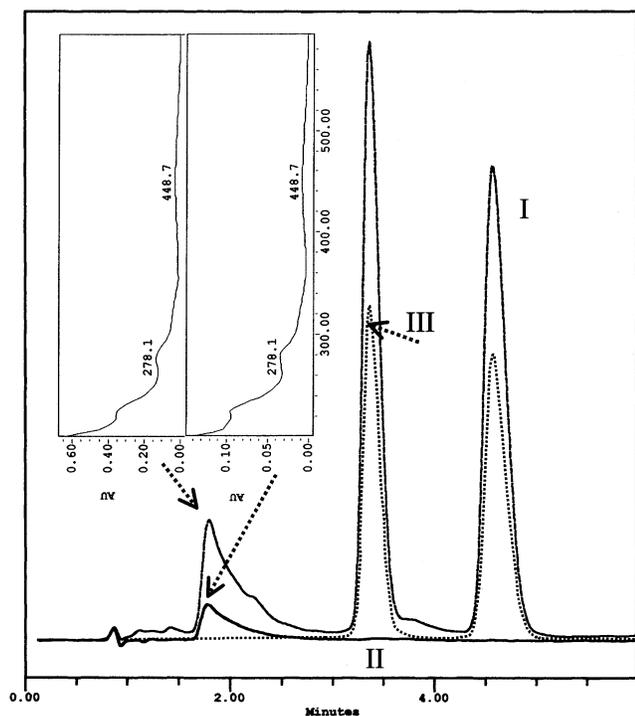


Figure 5. 480 nm Chromatograms of a typical Fe-EDDHA commercial chelate (I), Fe-*o,p*-EDDHA (II), and standard Fe-*o,o*-EDDHA (III). UV-visible spectra for peaks at 1.78 min are also included for I and II.

by the Fe–phenolate bond and there are two of these bonds in the structure of the *o,o*-EDDHA/ Fe^{3+} complex (**2** in **Figure 1**). The value of ϵ obtained for the *o,p*-EDDHA/ Fe^{3+} is consistent with a structure in which only one Fe–phenolate bond is present.

Once the target compound was obtained, we used it as a standard in the analysis of commercial *o,o*-EDDHA iron chelates. The analysis of Fe^{3+} complexes of *o,p*-EDDHA was carried out by using the method of Lucena et al. (16) by isocratic ion-pair high-performance liquid chromatography. Chromatograms of *o,p*-EDDHA/ Fe^{3+} and of a commercial sample of EDDHA/ Fe^{3+} complexes at 480 nm are represented in **Figure 5**. The chromatogram of the commercial chelate (I) shows two peaks at 4.56 and 3.36 min respectively, corresponding to the *meso* and *racemic* *o,o*-EDDHA/ Fe^{3+} complexes (**2b** and **2a** in **Figure 1**). In addition, chromatogram I shows another broad peak at 1.78 min, which is also present in chromatogram II, corresponding to the pure *o,p*-EDDHA/ Fe^{3+} complex. In the chromatogram of II, the peaks of the *meso* and *racemic* isomers of *o,p*-EDDHA are not separated. In **Figure 5** it is also clear that the UV–visible spectra for the 1.78 min peaks are almost identical for both the *o,p*-EDDHA/ Fe^{3+} complex and the commercial product. From the comparison of the chromatograms we can conclude that *o,p*-EDDHA/ Fe^{3+} chelate is the main impurity present in commercial EDDHA iron chelates. This result is in good agreement with our previous speculations (16, 17), but this is the first time in which direct evidence of the

presence of *o,p*-EDDHA ferric complexes in commercial samples is reported. The quantitative determination of *o,p*-EDDHA/ Fe^{3+} present in commercial chelates is currently being studied in our laboratories.

In conclusion, we have developed two alternative routes to the synthesis of *o,p*-EDDHA and by extension to any other unsymmetrical structurally related chelating agents. To the best of our knowledge, this is the first synthesis of *o,p*-EDDHA reported in the literature. Furthermore, having obtained a pure sample of *o,p*-EDDHA we have been able to confirm unambiguously that *o,p*-EDDHA/ Fe^{3+} complex is actually present in considerable amounts in commercial samples of *o,o*-EDDHA iron chelates. The structure and stability, and the potential use of the chelates derived from *o,p*-EDDHA as a source of iron, are currently under investigation in our laboratories.

ABBREVIATIONS USED

o,o-EDDHA, ethylenediamine-*N,N'*bis(*o*-hydroxyphenylacetic) acid; *o,p*-EDDHA, ethylenediamine-*N*(*o*-hydroxyphenylacetic)-*N'*(*p*-hydroxyphenylacetic) acid.

Supporting Information Available: Full experimental details and spectroscopic and analytical data for the compounds in **Figures 2, 3, and 6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- (1) Mengel, K. *Iron Nutrition in Soils and Plants*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995.
- (2) Chen, Y.; Barak, P. Iron nutrition of plants in calcareous soils. *Adv. Agron.* **1982**, *35*, 217.
- (3) Norvell, W. A. Reactions of metal chelates in soils and nutrient solutions. In *Micronutrients in Agriculture*; Mortvedt, C., Shuman, W., Eds.; SSSA Book Series 4; Soil Science Society of America: Madison, WI, 1991; p 187.
- (4) Bailey, N. A.; Cummins, D.; McKenzie, E. D.; Worthington, J. M. Iron (III) compounds of phenolic ligands. The crystal and molecular structure of iron (III) compounds of the sexadentate ligand *N,N'*-ethylene-bis-*o*-hydroxyphenylglycine. *Inorg. Chim. Acta* **1981**, *50*, 111.
- (5) Bernauer, K. Diastereoisomerism and diastereoselectivity in metal complexes. *Topics Curr. Chem.* **1976**, *6*, 1.
- (6) Kroll, H.; Knell, M.; Powers, J.; Simonian, J. A phenolic analogue of ethylenediamine tetraacetic acid. *J. Am. Chem. Soc.* **1957**, *79*, 2024.
- (7) Frost, A. E.; Freedman, H. H. Addition of hydrogen cyanide to aromatic schiff bases. *J. Org. Chem.* **1959**, *24*, 1905.
- (8) Knell, M.; Kroll, H. Iron chelates of ethylene bis(alpha-imino-ortho-hydroxyphenylacetic acid) and method of overcoming deficiencies in growing plants therewith. U.S. Patent 2,921,847.
- (9) Petree, H. E.; Myatt, L.; Jelenevsky, A. M. Preparation of phenolic ethylenediaminepolycarboxylic acids. U.S. Patent 4,130,582.
- (10) Julien, J. A. L.; Aymard, A. Nouveau procédé de preparation de l'acide ethylenediamine *N,N'*-bis(*o*-hydroxyphénylacétique) et de dérivés de celui-ci. European Patent 0,331,556 A2.

- (11) Liu, G. C.; Wang, Y. M.; Jaw, T. S.; Chen, H. M.; Sheu, R. S. Fe(III)-EHPG and Fe(III)-5Br-EHPG as contrast agents in MRI: an animal study. *J. Formosan Med. Assoc.* **1993**, *92* (4), 359.
- (12) Martell, A. E.; Motekaitis, R. J.; Sun, Y.; Ma, R.; Welch, M. J.; Pjeau, T. New chelating agents suitable for the treatment of iron overload. *Inorg. Chim. Acta* **1999**, *91*, 238.
- (13) Barak, P.; Chen, Y. Determination of Fe-EDDHA in soils and fertilizers by anion exchange chromatography. *Soil Sci. Soc. Am. J.* **1987**, *51*, 893.
- (14) Deacon, M.; Smyth, M. R.; Tuinstra, L. G. M. Chromatographic separations of metal chelates present in commercial fertilizers. II. Development of an ion-pair chromatographic separation for the simultaneous determination of the Fe(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the Cu(II), Zn(II) and Mn(II) chelates of EDTA. *J. Chromatogr. A* **1994**, *659*, 349.
- (15) Lucena, J. J.; Barak, P.; Hernández-Apaolaza, L. Isocratic iron-pair high liquid chromatographic method for the determination of various iron (III) chelates. *J. Chromatogr. A* **1996**, *727*, 349.
- (16) Hernández-Apaolaza, L.; Barak, P.; Lucena, J. J. Chromatographic determination of commercial Fe(III) chelates of ethylenediaminetetraacetic acid, ethylenediamine di(*o*-hydroxyphenylacetic) acid and ethylenediamine di(*o*-hydroxy-*p*-methyl-phenylacetic) acid. *J. Chromatogr. A* **1997**, *789*, 453.
- (17) Cremonini, M. A.; Alvarez-Fernandez, A.; Lucena, J. J.; Rombola, A.; Marangoni, B.; Placucci, G. J. Nuclear magnetic resonance analysis of iron ligand EDDHA employed in fertilizers. *J. Agric. Food Chem.* **2001**, *49*, 3527.
- (18) Stein, G. A.; Bronner, H. A.; Pfister, K. α -Methyl α -amino acids. II. Derivatives of DL-phenylalanine. *J. Am. Chem. Soc.* **1955**, *77*, 700.
- (19) Wang, Q. X.; Phanstiel, O. IV. The total synthesis of acinetoferrin. *J. Org. Chem.* **1998**, *63*, 1491.
- (20) Savitzky, A.; Golay, J. E. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* **1964**, *36*, 1627.
- (21) Myers, A. G.; Kung, D. W.; Zhong, B. Observations concerning the existence and reactivity of free α -amino aldehydes as chemical intermediates. Evidence for epimerization-free adduct formation with various nucleophiles. *J. Am. Chem. Soc.* **2000**, *122*, 3236.
- (22) Vachal, P.; Jacobsen, E. N. Enantioselective catalytic addition of HCN to ketoimines. Catalytic synthesis of quaternary amino acids. *Org. Lett.* **2000**, *2*, 867.
- (23) Sigman, M. S.; Vachal, P.; Jacobsen, E. N. A general catalyst for the asymmetric Strecker reaction. *Angew. Chem., Int. Ed.* **2000**, *39*, 1279.
- (24) Sen, S. E.; Roach, S. L.; Boggs, J. K.; Ewing, G. J.; Magrath, J. Ferric chloride hexahydrate. A mild hydrolytic agent for the deprotection of acetals. *J. Org. Chem.* **1997**, *62*, 6684.
- (25) Cainelli, G.; Giacomini, D.; Treré, A.; Galletti, P. Acyclic stereocontrol in the addition of trimethylsilyl cyanide to *N*-substituted imines of (2*S*)-lactic aldehyde. *Tetrahedron: Asymmetry* **1995**, *6*, 1593.
- (26) Ahrland, S.; Dahlgren, A.; Persson, I. Stabilities and hydrolysis of some iron(III) and manganese(III) complexes with chelating ligands. *Acta Agric. Scand.* **1990**, *40*, 101.

Received for review June 7, 2002. Revised manuscript received July 26, 2002. Accepted July 26, 2002. Support for this work under Grant 2FD97-0314-CO2 from the CICYT (MEC-Spain) and the European Commission is acknowledged. Grants to P. Ramírez (CAM), F. Yunta (MEC), and S. García-Marco (CAM) are also gratefully acknowledged.

JF025727G